Applicability of the Project at an Industrial Scale

As an effort to understand the applicability of our project on an industrial scale, I visited a bioreactor facility in the Biotech Park, Lucknow, Uttar Pradesh. As of July, 2016, the government has installed nineteen Biotech Parks all over the country. These parks provide support to small scale startups by providing them bioreactors so that their product can be synthesised on a small scale. The bioreactors in the Lucknow facility were used mainly for production of bio-pesticides and bio-fertilizers.

Bioreactors of three different capacities have been installed. The smallest one is of the capacity of two litres. This bioreactor is used mainly for R&D purpose. This bioreactor was quite sophisticated as different parameters, like temperature, aeration, pH, etc. As the volume of bioreactors increases, we lose the precision with which these parameters can be controlled.

The next two capacities of bioreactors were five hundred and two thousand litres. They both had the same working more or less. Before use, these bioreactors are sterilized by passing steam through them for two hours. It would generally take about a day for these bioreactors to be cooled to a usable temperature. The media would then be added through an opening on the top. The inoculum is added from the funnel on the top of the bioreactor. The funnel is sterilised by washing it with ethanol and then setting it on fire. The inoculum is then added from outside quickly in order to prevent contamination. Any more additions, if required, is done from these openings at the top. There is one inlet for fresh air to enter the bioreactor. To maintain sterility, air is passed through a 0.22 micron filter. Agitation of the culture is achieved by rotating blades. The growth rate of the culture is largely controlled by the aeration and agitation. Stationary phase can be achieved in three days to a week. There is an outlet for the culture at the bottom of the bioreactor.

Picture 1: The top of a 500 l bioreactor. The inoculum is added from the funnel and the media is added from the circular opening. The rotor for the agitator blades can be seen at the top.

During the course of the bacterial growth, they take out small volume samples to check for the cell density, pH and contamination. They do not measure the optical density as it is not a good measure of the cell density. They plate the culture and count CFUs. On plating, they also get to know about contamination by looking at the colonies. These bioreactors do not require strict pH and cell density monitoring. However, the scientists there told me that bigger bioreactors require monitoring and samples are taken out periodically.

Picture 2: The outlet is at the bottom of the bioreactor. Samples are taken out from this outlet to check for contamination and to monitor cell densities.

In case of contamination, the whole culture is discarded and the bioreactor is sterilised thoroughly. The scientists could not tell me the frequency of contamination as the bioreactor had not been used many times.

The microbes grown in these bioreactors are *Trichoderma harzianum and Pseudomonas fluorescens.* These microbes secrete enzymes like cellulase which breaks the cell wall of other microbes. Since, they wish to sell these bacterial cultures as products, they don't need to induce or clarify the culture. Hence, I did not find any centrifuges here.

Picture 3: The products manufactured at Biotech Park. These products include bio-pesticides, bio-fertilizers and plant extracts.

Since the use of GMO in the agricultural industry is prohibited by law, these bioreactors are not designed for induction or clarification of culture and further downstream processes. However, if

need be, using our idea GMO can be grown in these bioreactors. Also our idea can reduce the timescales on which these bioreactors work if they need to extract some by-products from bacteria. Our project can fit in several ways. We will discuss on this further.